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COLORIMETRIC METHOD FOR ESTIMATION OF PROTEOSE-PEPTONE IN MILK[1]

ABSTRACT

A colorimetric method for the estimation of proteose-peptone in milk has been described. The different conditions studied, such as absorption maximum, color-concentration proportionality, added recovery and comparison of nitrogen value with colorimetric value for proteose-peptone, satisfy the requirements for colorimetric determination. The advantages offered in simplicity and sensitivity of the reaction recommend it. The average values for proteose-peptone in cow and buffalo milks were 220.9 ± 9.9 mg and 172.1 ± 7.6 mg per 100 ml of milk, respectively.

Proteose-peptone is a minor constituent present in milk. To elucidate the exact role of this component in milk, investigations were undertaken in this laboratory recently. Such a study has evoked our interest, firstly to evolve a simple method for its estimation in milk which will enable us to analyze a reasonably good number of samples every day.

The existing procedures (9, 10) for analysis of proteose-peptone in milk are based on the chemical fractionation of the protein components in milk, followed by subsequent estimation of nitrogen by the Kjeldahl method (5), which is time-consuming and cumbersome.

Folin phenol reagent has been widely used for determination of proteins (2, 7) and has considerable merit for its application in such estimations. Since this estimation is based on reaction of aromatic amino acids, which are also present in proteose-peptone (3, 12), it was thought worthwhile to examine the suitability of the method of Lowry et al. (7) for estimating proteose-peptone. This method for milk protein estimation has been used by others (6). The present report is the result of an attempt to delineate the standard conditions needed for the test.

MATERIALS AND METHODS

Reagents. Folin-phenol-reagent (FPR) was prepared by the procedure of Folin and Ciocalteu (2) and all other reagents used in the estimation of proteose-peptone were prepared according to Lowry et al. (7). Reference proteose-peptone standard used was a dry sample isolated and purified from milk according to Aschaffenburg (1) with suitable modification (3). All chemicals used were of analytical grade.

Procedure. Twenty milliliters of milk diluted to 60 ml with water was heated in boiling water bath for 15 min (the volume of milk taken and the heating period were fixed on the basis of preliminary studies). On cooling the contents to room temperature, 2 ml each of 10% acetic acid and 1 N sodium acetate was added and finally volume made to 100 ml with water and mixed thoroughly. The precipitated proteins were filtered using Whatman no. 42 paper and the filtrate thus obtained was diluted ten times with water (acetic acid filtrate).

Another 10-ml aliquot of the original filtrate was treated with 10 ml of 16% trichloroacetic acid (TCA) and filtered as above and finally diluted five times with 0.7% sodium carbonate to bring the pH to 4.5 (TCA filtrate); 0.5 ml aliquot each of acetic acid filtrate and TCA filtrate was then used to develop color with FPR, according to Lowry et al. (7). The color was finally read at 750 mμ in Beckman DU spectrophotometer, using cell of 1-cm light path.[2] The proteose-peptone (PP) content was calculated from the difference in the readings due to acetic acid filtrate and TCA filtrate, using a standard curve prepared from PP[3] as shown in Figure 1. The slope of such curve was examined separately, using PP samples isolated from cow and buffalo milks, and was

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[2] A Klett-Summerson photoelectric colorimeter can as well be used for reading the sample with a No. 66 filter. Samples do not need any further treatment for such purpose.

[3] A separate standard curve with proteose-peptone has to be prepared when the Klett-Summerson colorimeter is used.

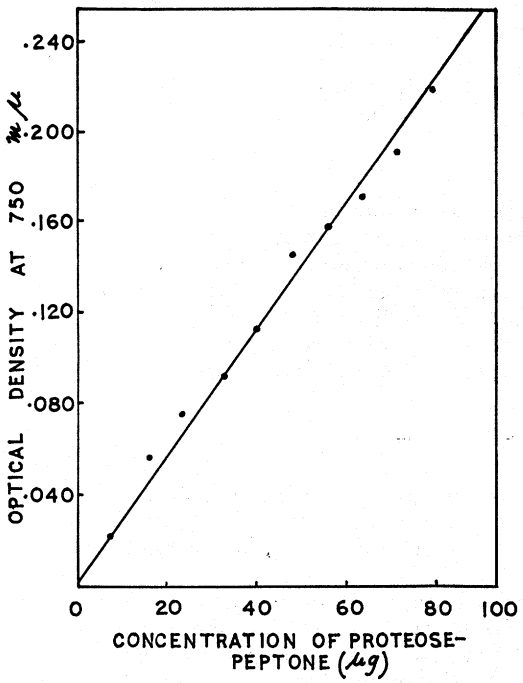

FIG. 1. Standard curve for proteose-peptone, showing the color-concentration proportionality.

observed to be 1.4281 and similar. For such reason, only one such curve was used for calculations.

The nitrogen content of the filtrates was determined by the micro-Kjeldahl method (5).

RESULTS AND DISCUSSION

To establish the applicability of the present procedure, the estimation of PP in milk was carried out at different conditions.

(a) *Recovery of added proteose-peptone from milk*

Proteose-peptone was added at the rate of 30 and 45 mg to 20 ml of milk and recovery computed. It is quite apparent from the results shown in Table 1 that the recovery of the added PP is satisfactory. On the basis of these results, it is also apparent that PP added to milk has not suffered destruction in any of the steps involved in this procedure.

TABLE 1
Recovery of added proteose-peptone from milk

Proteose-peptone added (mg)[a]	Proteose-peptone recovered (mg)	Per cent recovery
30	28.6	95.3
30	32.2	107.3
45	50.6	112.4
45	46.2	102.7
45	47.6	105.8

[a] Proteose-peptone isolated from milk was used for such study.

(b) *Comparison of proteose-peptone contents as evaluated by the present colorimetric method and by nitrogen estimation*

The nitrogen content of the isolated PP sample was first determined and the values for six such estimations was observed to range between 12.0-12.5%. On the basis of these results, it was decided to use a factor of 8.15 (100/12.26) to obtain the PP present in milk by Rowland's (8, 9) procedure. Our values for nitrogen in PP seem to be higher than reported values by Weinstein et al. (12) (10%), but lower than Aschaffenburg's (1) value (13.9%).

Table 2 represents results of some milk sample analyzed for PP by both nitrogen methods (8, 9) and also by the present colorimetric method. The agreement between the values justifies the adoption of the colorimetric method. It can also be noted that using 6.38 as a factor, one would naturally record a lower value, as done by previous workers (1, 4, 9-11), than the real content.

(c) *Estimation of proteose-peptone in milk samples from cow and buffalo, using the colorimetric procedure*

Milk from individual milch animals of different breeds of cow and buffalo was collected for analysis. The time lapse between collection of milk and estimation of PP was 2 or 3 hr. Results of these analyses are presented in Table 3. Results indicate that the PP content of milk varies from sample to sample and ranges between 51-395 mg per 100 ml of milk. However, it has been observed that the maximum number of samples fall between 100 to 250 mg per 100 ml of milk. Average values for the different breeds of cow milk samples did exhibit some difference in the PP content. Such values for PP in cow milk is 220.9 ± 9.9 mg (an average

TABLE 2
Proteose-peptone content of milk as estimated by Kjeldahl and colorimetric methods

Source of milk	Breed	Proteose-peptone content (mg/100 ml)	
		Colorimetric method	Kjeldahl (N × 8.15) method
Cow	Sahiwal	272	244
	Red Sindhi	210	200
	Tharparkar	200	195
Buffalo	Murrah	238	240

TABLE 3

Concentration of proteose-peptone in milk from cow and buffalo

Animal	Breed	No. of samples [a]	Proteose-peptone content (mg/100 ml) Range	Proteose-peptone content (mg/100 ml) Average	Statistical "t" value between cow and buffalo
Cow	Tharparkar	28	103–395	247.1 ± 16.1 [b]	
	Red Sindhi	6	134–295	224.0 ± 24.7	3.41 [c]
	Sahiwal	36	51–391	200.1 ± 13.3	
Buffalo	Murrah	40	82–282	172.1 ± 7.6	

[a] Number of samples analyzed was dependent on the availability of milk from the individual breeds, for which reason same number of each breed could not be analyzed.
[b] Mean ± standard error.
[c] Significant at 1% level ($P < 0.01$).

for 70 samples), whereas for buffalo milk the value is 172.1 ± 7.6 mg (an average for 40 samples). Statistical analysis has revealed that the average difference between cow and buffalo PP values is highly significant ($P < 0.01$). The wide range of variation in the PP content in milk as observed by us agrees well with the range obtained by Shahani and Sommer (11). Considering their average value for PP nitrogen, i.e., 23 mg/100 ml, and on conversion with our factor of 8.15, one gets a value of 218.5 mg, which approaches our result. This further strengthens the applicability of the present colorimetric method for evaluation of PP in milk.

Such wide variation in the PP content of milk can be attributed to the stage of lactation of the animal, as indicated by Ghosh and Anantakrishnan (4). However, the seasonal and feed effects cannot be ruled out at present and will be the subject matter of subsequent studies.

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